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A new gene in *A. rubens*: A sea star Ig kappa gene

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ABSTRACT

The sea star *Asterias rubens* reacts specifically to the antigen:HRP (horse-radish peroxydase) and produces an antibody anti-HRP. We previously identified a candidate Ig kappa gene corresponding to this manuscript. We show now the gene referred to as: "sea star Ig kappa gene in its specificity".

Introduction

A first experiment which was fundamental, described in 1973, discusses the immune response to various antigens of an asterid: *Asterina gibbosa*. It indicated (Leclerc, 1973), more exactly, the specific immuno-cytochemical response to HRP (horse-radish peroxydase) of the sea star. The general idea that emerged from the experiments made in our laboratories was that Echinodermata (Leclerc and Brillouet, 1981; Delmotte et al., 1986; Leclerc, 2012), as exemplified by sea stars (*Asterina gibbosa* and *Asterias rubens*), possessed an immune system able to mount cellular and humoral-specific responses after stimulation with a foreign antigen. In 2011 sea star antibody was shown to correlate to Ig kappa genes (Leclerc et al., 2011) and in 2013 a "true" candidate Ig kappa gene was found (Leclerc et al., 2013). Then, it was shown as a "true" gene from this candidate.

Here we report the sequence and deduced amino-acid composition of the sea star Ig kappa gene.

Materials and methods

RNA was extracted using Trizol (Invitrogen) according to the manufacturer's instructions. We used the experimental protocol concerning the SMART kit PCR cDNA Synthesis (Clontech) on the candidate gene

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SMART_956.ab1:
5'tGACTGCTGCTATGCGTGGCAACATGGCGTCTCTATGGATGTTCTTCTTTGTCgTGGGGA
TAACTTTACAACGGAGTTTGGCGATTTACACGTTTCGCGAGCAACCGTCGGACACTAGC
GCGTTGCAGGGGAGCACAGTGGTGCTTCACTGCTCCGTTGAGCAGTACATAAACACCAC
GGCCATCGTTTGGTGGAGCCGTGACTCGGTCATCAGCCACAACAAAGACCTGAAACTG
TCCAGTCTAAACACCGACCAGCTCCAAAGGTACTCGATTTCAGGCGACGCATCTCGGGG
GGAATTCAACCTTAAAATAGTGAACTTTACCGNCACAGACGCCGCCAGTTACCGCTGTC
AGATGTTTGCGA3'

Fig. 1. DNA sequence of sea star Ig kappa gene. The SMART 956 ab 1. 366 bp sequence is shown. Next the deduced amino-acid sequence encoded by the DNA is a 118 aa protein shown in Fig. 2.

cDNA sequence (Leclerc et al., 2013) from *A. rubens*, as following. This SMART was specifically designed for the recovery of the target gene.

5′CAGTCATTAAAAGGACATGATAATTTCGGACCGGGTCTTTAATATTACAATGACTGCTGCTATGCGTGGC
AACATGGCGTCTCTATGGATGTTCTTCTTTGTCGTGGGGATAACTTTACAACGGAGTTTGGCGATTTACA
CGTTTCGCGAGCAACCGTCGGACACTAGCGCGTTGCAGGGGAGCACAGTGGTGCTTCACTGCTCCGTTGA
GCAGTACATAAACACCACGGCCATCGTTTGGTGGAGCCGTGACTCGGTCATCAGCCACAACAAAGACCTG
AAACTGTCCAGTCTAAACACCGACCAGCTCCAAAGGTACTCGATTTCAGGCGACGCATCTCGGGGGGAAT
TCAACCTTAGAATAGTGAACTTTACCGCCACAGACGCCGCCAGTTACCGCTGTCAGATGTTTGCG3′

We purified the polyA fraction, according a specific kit and generated the c DNA with an oligodT.

A non-specific amplification was performed and followed by a specific one with an oligo 956 forward (5′–3′) 5′-CAGATTCAGAAACACATGTATTTCC-3′ and then an oligo 957 reverse (5′–3′) 5′-TTTAGCATGGCATG TAAAGACACC-3′, always requested for this experiment (Clontech).

The PCR products showed, in agarose gel, many bands for the negative control, and one band (400 bp) for the specific PCR. This last was purified and sequenced on Illumina's GSII platform sequencing. Experiments, in duplicate, were performed.

Results

The results show that the revealed gene, after sequencing, shows only little gene described in the previous paper (Leclerc et al., 2013) (Swissprot database.)

It is enough to decipher this gene to realize it; here is the sequence as shown in Fig. 1.

Discussion and conclusion

Based on Sander's review (Sander and Schneider, 1991), we propose that this gene relates to immunoglobulins as it contains 2 typical cysteines of the domains of Ig, without being able to assert, at the moment, if it is about a heavy or light chain of immunoglobulins. A single indication (Delmotte et al., 1986) favors the light chain due to the observed molecular weight (30,000 Da: Delmotte et al., 1986).Otherwise “this sea star antibody” corresponds to HRP antigen. Another antigen such as hapten (Leclerc and Brillouet, 1981) could produce another type of “antibody” Currently, we don't know, at the moment, if there are several types of antibody in the sea star *A. rubens*. What we are sure, on the other hand (because there is a gene (the sea star Ig kappa)), the function of which is the one of the defenses of the sea star against the immunopathogenicity attacks; the HRP, in the present case. In terms of amino-acid, our gene could present 118 amino-acids as shown in Fig. 2. This is slightly smaller than the true Ig kappa region V-IV S107B which is reported to be 129 amino-acids found in mammals.

MRGNMASLWM FFFVVGITLQ RSLAIYTFRE QPSDTSALQG STVVLHCSVE QYINTTAIVW

WSRDSVISHN KDLKLSSLNT DQLQRYSISG DASRGEFNLK IVNFTXTDAA SYRCQMFA

Fig. 2. Sea star Ig kappa gene including amino-acid sequence (118 aa).

In conclusion, this antibody gene of sea star calls back the mouse Ig kappa region V-IV S107B precursor gene but is different from it by the number of amino-acids (118 instead of 129 aa).

Our data contributes to knowledge the molecular and genetic bases of non-self recognition by invertebrates which will allow further comprehension of the evolution of MHC antigens and immunoglobulins.

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